R E M A R K S

Upon entry of the above amendment claims 1-26, 30-33 and 37-58 will be pending in the above-captioned application.

The amendment to the introductory part of claim 1 is made to render the claim clearer and to ensure conformity with the wording "ribonucleic acids" in steps e) and f). Support for the amendment in step c) of claim 1 is found on page 3, lines 10-21, page 5, lines 5-25 of the specification, namely, that detailed knowledge of cellular mechanisms underlying the preselected phenotypic trait is unnecessary. Finally, in step f) it has been specified that the use of the identified RNA and peptides as "fishing hooks" involves both isolation and *identification* of ligand molecules.

The amendment to claim 26 is made to specify that the alteration of the preselected phenotypic trait is up- or down-regulation of expression of a cell-surface molecule. Support for this aspect is found at page 9, lines 13-25 of the specification. It should be clear that the terms "up-regulation" and "down-regulation" imply that the cell-surface molecule is expressed on the surface in both transduced and non-transduced cells, cf. especially lines 12-15.

Claim 32 has been amended in a manner consistent with claim 1, specifically, in the introductory part and in step f). Further, step c) has been amended by substituting the expression "...capture of the expressed ribonucleic acid(s) or peptide(s) with a ligand..." with the following: "...detection of interaction between the expressed ribonucleic acid(s) or peptides(s) and a preselected enzyme or receptor..." . Support for this wording is found in the specification on page 3, lines 16-21, as well as on page 5, lines

6-13. As will be apparent from the references cited in the specification, a "receptor" as referred to on page 1, line 36, in the specification, is an alternative to the term "binding partner": In the references mentioned, the ligands identified bind to antibodies and streptavidin, respectively, and from the reference by Cull et al it is clear that this broad meaning of the term "receptor" is accepted in the art, cf., e.g., the Abstract in Cull et al where it is mentioned that an antibody is used as a model receptor.

Claim 33 has been amended in a manner consistent with claim 1, specifically, the introductory part and in step f). Further, in step c) the wording "...is ascribable to the expressed ribonucleic acid(s) or peptide(s) affecting biological functions of the cell which have influence on the preselected phenotypic trait..." has been replaced with the wording "...indicates that ribonucleic acid(s) or peptide(s) encoded by the vector DNA affect(s) biological functions in the cell and thereby effects the alteration of the preselected phenotypic trait..." Support for this wording can be found in numerous locations in the specification, for example, page 3, lines 34-38.

Claim 41 has been amended by exchanging the wording "...ligand molecule..." with "...cellular target protein...". Support for this wording can be found throughout the specification, for example, page 4 line 5.

Claim 42 has been amended in a manner consistent with claim 1, specifically, the introductory part and in step f). Further, in step c) the wording "...altered a preselected phenotypic trait , said phenotypic trait being an observable characteristic of the identical eukaryotic cells prior to and after transduction..." has

been exchanged with the wording "...up-regulated or down-regulated a preselected biological effect...". Support for this wording can be found on page 5, line 24, and in Example 3.

New claim 43 is directed to a method for identification of cellular target proteins, similar in scope to original claim 1.

New claims 44-47 include limitations corresponding to those of claims 33, 42, 1, and 26, respectively.

New claims 48-52 include the limitation that the eukaryotic cells are mammalian. Support for this aspect of the invention is found at page 4, line 15, of the specification.

New claims 53-57 specify that the identical eukaryotic cells are from a cell clone or a cell line. Support for this aspect of the invention is found at page 9, lines 13 and 36 of the specification.

Finally, new claim 58 relates to an improved drug development method, which includes use of a cellular target protein identified by means of the method of claim 43. Support for this aspect of the invention can be found in the specification on page 4, lines 4-9, page 7, lines 4-6, page 10, lines 3-5, and page 17, lines 8-11.

NON-ART BASED REJECTIONS

(1) The Examiner has rejected claims 32-34 under 35 U.S.C. § 112, first paragraph.

First, claim 34 has been deleted and therefore the rejection as it pertains to this claim is moot.

With respect to claim 32, the Examiner's rejection is due to the presence of the language "...wherein the screening method is different from capture of the expressed ribonucleic acid(s) or peptide(s) with a ligand..." and it is held that this limitation

introduces new matter. Applicants submit that in view of the above amendment to claim 32 and submissions below, this rejection should be withdrawn.

On page 5 in the present specification, first full paragraph, it is clearly indicated that all known prior art methods rely on the availability of an enzyme or receptor in relatively pure form in order to enable selection of a ligand encoded by inserted random DNA. This, taken together with the discussion of prior art methods on pages 2 and 3 in the specification, clearly indicates to the skilled person that the present invention is distinguished from the prior art by, i.e., its lack of need for a recognized binding partner (i.e., a ligand) to the expression product of the transduced DNA. In fact, the use of a specific and well-characterized binding partner is clearly indicated in the present specification as being a problem associated with the prior art methods when compared to the present invention, cf. page 5, lines 7-25. Reconsideration and withdrawal of the rejection to claim 33 is respectfully requested.

The Examiner's rejection of claim 33 is due to the presence of the limitation "...where alteration of the preselected phenotypic trait is ascribable to the expressed ribonucleic acid(s) or peptide(s) affecting biological functions of the cell which have influence on the preselected phenotypic trait..." and it is held that this language introduces new matter.

This rejection is respectfully traversed. The paragraph bridging pages 3 and 4 clearly indicates that some of the expression products of the random DNA effectively transduced into the cells will affect important biological functions of the cell and that cells which change phenotype due to the presence of such

expression products can be isolated. On page 7, last paragraph, a similar description is given for the situation where RNA transcripts of the DNA transduced into the cells interact with the cellular biochemistry in a way analogous to that seen when expressing aptamer libraries. It would thus be clear to the skilled person that the present invention is capable of identifying RNA or peptides which are expression products of randomized DNA and which interact with the cellular biology so as to induce a change in the cell's phenotype. Also, Examples 1-4 all describe embodiments where the random expression products induce phenotypic changes in the transcribed cells and these changes are all the result of interactions between the biochemistry of the transduced cells and the expression products. This is in contrast to the Kay *et al* disclosure, where the phenotypic changes (binding of the expression product to a preselected ligand) cannot in any way be ascribed to the expression product affecting the biochemistry of the cell.

In any event, Applicants have amended claim 33 to specifically recite that alteration of the preselected phenotype "...indicates that ribonucleic acid(s) or peptide(s) encoded by the vector DNA affect(s) biological functions in the cell and thereby effects the alteration of the preselected phenotypic trait...".

Applicants submit that the limitation set forth in claim 33 very accurately summarizes the main characteristic of the present invention, namely, that it enables the identification of expression products which *indirectly* alter the phenotype of a cell due to, for example, intracellular interactions between some of the random expression products and the cell's biological functions. Reconsideration and withdrawal of the rejection is respectfully requested.

(2) The Examiner has rejected claims 33 and 34 under 35 U.S.C. § 112, second paragraph.

The rejection of claim 34 is moot in view of the deletion of this claim.

As for claim 33, Applicants' submit that, in view of the above-discussed amendment to claim 33, the rejection is overcome. Claim 33 now clearly specifies that an observed change in phenotype indicates that the expression products are biologically active in the cell and thereby effect the phenotypic change which is observed. The skilled person would, based on the present specification, readily appreciate that this feature of the claim reflects the *type* of phenotypic alteration which is screened for. For instance, if the phenotypic change is up- or down-regulation of a surface molecule which is present in transduced as well as non-transduced cells it should be clear that this indicates that the expression product exerts a biological effect in the cell. Reconsideration and withdrawal of the rejection is respectfully requested.

ART BASED REJECTIONS

(1) The Examiner has maintained the rejection under 35 U.S.C. § 102(e) of claims 1-2, 4-5, 8-11, 15-16, 20, 22-24, 30-31, 35-37 and 39-42 as being anticipated by Kay et al.

The Sigmund C reference and appropriateness of the rejection under §102(e):

It is Applicants' submission that the use of the Sigmund reference under § 102(e) is improper. A secondary reference may be used in making a § 102 rejection only to (1) prove the primary reference contains an "enabling disclosure", (2) explain the meaning of a term used in the primary reference, or (3) to show that a characteristic not disclosed in the primary reference is inherent.

The Examiner has argued the appropriateness of the secondary reference in order to show that the Kay et al reference contains enabling disclosure/operability. The Examiner has referred to In re Donohue, 226 USPQ 619 (Fed. Cir. 1985) and has stated that "...a reference contains enabling disclosure if the public was in possession of the claimed invention before the date of invention. Such possession is effected if one of ordinary skill in the art could have combined the publications' description of the invention with his [her] own knowledge to make the claimed invention." However, Applicants' argument is that Kay et al fails to teach each and every element of the claimed invention, specifically that Kay is silent as to the step of transducing the vectors into a number of identical eukaryotic cells in such a way as to effect a one cell one gene transduction. The Examiner has admitted that such a step is not taught by Kay et al. According to In re Donohue, "*If the reference teaches every claimed element of the article*, secondary evidence, such as other patents or publications, can be cited to show public possession of the method of making/or using." Since Kay et al does not teach every claimed element the use of a secondary reference is therefore not proper.

The Examiner has argued that although such a step is not taught, Kay et al does teach that the method can be practiced with viral vectors in a eukaryotic host. While Applicants agree that the specification of Kay et al mentions the possibility of using viral vectors such as retrovirus and therefore Kay et al (at least formally) discloses transduction of cells. However, since Kay et al does not indicate that it is of interest to effect transduction of eukaryotic cells so as to obtain a "one cell - one gene" transduction, it is improper to combine Sigmund C into the teaching of Kay et al. Only in the case where Kay et al had implicitly or explicitly expressed that there is a need to obtain a "one cell - one gene" effect, it would have been proper to combine the teaching of Sigmund C into the teaching of Kay et al for the purposes or §102(e). However, Kay et al does not indicate any such need. On the contrary it is stated that eukaryotic cells could be inserted into eukaryotic cells by means of electroporation (cf. the paragraph bridging columns 28 and 29), a technique which would not provide for a "one cell - one gene" effect in eukaryotic cells unless very special measures were undertaken.

Therefore, it is clear that Kay et al does not anticipate the presently claimed invention, and if the Examiner intends to maintain the citation of Sigmund C, it is respectfully submitted that this should be done in the context of 35 U.S.C. § 103. Furthermore, Sigmund C will be required to make complete the other rejections under §103 which rely on Kay et al as the primary reference.

The present invention

Prior to addressing the Examiner's rejection, in the event it is still deemed by the Examiner to be proper in view of the above comments, Applicants present a discussion of the present invention to assist the Examiner, as follows.

The present invention provides for a method which identifies *biologically active* RNA or peptides or cellular ligands to such *biologically active* RNA or peptides. It is apparent from the specification that the term "biologically active" in the present context means that the RNA or peptides affects biological functions in living cells so that their phenotype is changed.

One advantage of the claimed method over prior art methods for identifying potentially interesting biomolecules is that the *biological activity* of RNA or a peptide is established prior to identification of cellular molecules interacting therewith. Prior art methods for biomolecule identification (such as the one disclosed in Kay et al) rely on capture of library peptides with a known interaction partner (e.g. an antibody). However, such prior art methods do not necessarily identify biomolecules with a *biological activity*. All which can be said about a library member which is identified according to the method of Kay et al is that under the conditions of the chosen capture assay, the library member has exhibited an ability to bind to the chosen binding partner used in the assay. Whether or not the identified biomolecule is capable of exerting biological activity in a cell has to be tested in a subsequent assay and whether or not such a biological activity is agonistic or antagonistic also have to be established later. Reference is made to the discussion in the present specification on page 5, lines 13-17.

Furthermore, the prior art methods require that a known interaction with a known target molecule is detected, whereas this is not the case for the present method; in fact, one important feature of the invention is its ability to identify hitherto unknown target molecules in cells. This feature has been made the subject matter of new claim 43. Hence, the presently claimed method renders unnecessary the availability of an assay detecting a interaction with a known partner for the RNA or peptides and the screening methods used in the method of the invention are deliberately chosen so as to avoid detection of such known partners.

The issue of "biological activity" is very important. A peptide which is identified by the presently claimed method *is* biologically active, simply because it is the biological activity which is screened for. For a biomolecule to be biologically active, it has to appear in a sufficient concentration in the cell, it has to be active under the particular environmental conditions prevailing in the relevant cellular compartment, and it has to be capable of interfering with target molecules inside the cell. Only the latter of these features can be reasonably determined by using the prior art methods cited by the Examiner.

It should also be noted that it cannot in any way be concluded that a peptide is biologically active in a mammalian cell if it has been shown earlier that this is the case in, e.g., a bacterium or even a lower eukaryotic cell such as a yeast cell - the environment inside mammalian cells is not at all comparable to the environment in such lower cells and this can have very marked influence on the activity of such a peptide.

The Examiner's rejection

Addressing the Examiner's specific rejection, Applicants' submit that first, claim 1 has been amended to specifically recite that the screening method used does not require knowledge of 1) chains of mechanisms in the cell, 2) enzymes in the cell, 3) signalling pathways in the cell, or 4) receptors in the cell which generate the preselected phenotypic trait. Nowhere in Kay et al can a disclosure of such a limitation be found. In fact, the screening methods according to Kay et al require full control over the provision of the expression product which is ultimately captured with a immobilised binding partner, since it is the direct binding properties of the expression product which constitute the "phenotypic trait".

Second, as discussed above, the feature of the invention that transduction *must* result in the provision of transduced cells with either one single expression product or a limited number of expression products from the transduction vector is disclosed nowhere in Kay et al and nowhere is any incentive to obtain this effect provided for the skilled person - hence, to include the teachings of Sigmund C into the teachings of Kay et al seems to be an improper widening of the teachings of Kay et al.

Third, claim 42 now includes the limitation that the screening identifies up- or down-regulation of a preselected biological effect. As discussed above, the terms "up-regulation" and "down-regulation" imply that the biological effect exist both in transduced and non-transduced cells, cf. the text on page 9, first full paragraph. Again, this clearly distinguishes the claimed subject matter from the Kay et al disclosure, because the

"biological effect" which is screened for in Kay et al is one which is not present in the cells prior to transformation.

It should also be noted that the present claims recite the identification of "biologically active" RNA or peptides. It should be borne in mind that the method described in Kay et al does not identify biologically active RNA or peptides, *i.e.* RNA or peptides which affect biological functions in a eukaryotic cell. What is described in Kay et al is a method for identifying binding partners which may or may not be biologically active, cf. the discussion on page 5, lines 7-17 in the present specification. The present method implicitly identifies biologically active expression products since it is the biological effect which is the object of the screening method.

In view of the above submissions, reconsideration and withdrawal of the rejection is respectfully requested.

(2) The Examiner has rejected claims 6, 12-14, 17-19 and 21 under 35 U.S.C. § 103(a) as being unpatentable over Kay et al in view of Burke et al and Wong et al. The rejection is respectfully traversed.

First, Applicants submit that for reasons already given with respect to the Kay et al reference, the claimed invention is neither taught nor suggested by the cited art.

Second, none of the references Burke et al and Wong et al provide the skilled person with the essential features of claim 1, *i.e.* a screening method which fulfils the criteria mentioned in claim 1 or an incentive to effect the "one cell-one gene" transduction of eukaryotic cells which is required by claim 1. Finally, neither Burke et al nor Wong *et* al provide a teaching which would ensure that the method of Kay et al results in the

identification of a biologically active molecule. Hence, the combination of the 3 references does not lead to the claimed invention. Withdrawal of the rejection is respectfully requested.

(3) The Examiner has rejected claims 25 and 26 under 35 USC 103 (a) as being unpatentable over the above cited references, further in view of Stemmer et al.

First of all, it should be noted that Stemmer et al is not a proper reference. Stemmer et al issued on 3 November 1998, *i.e.* 3 years and 5 months after the filing of the priority application underlying the present application. The § 102(e) date is, according to the front page of US 5,830,721, 4 March 1996 which is approximately 9 months after the priority date of the present invention. Finally, the PCT application from which Stemmer et al is derived was published on 24 August 1995 which is 2 months and 22 days after the priority date of the present application. Therefore, Stemmer et al does in our opinion not constitute citable prior art against the present invention, since the subject matter of both of claims 25 and 26 also is part of the priority application, cf. Examples 2 and 3 in the priority document.

Furthermore, as is the case for Burke et al and Wong et al, Stemmer et al does not teach the "one cell - one gene" aspect of the present invention, and neither is the screening method as defined in claim 1 taught. It is, for example, noted that Stemmer *et al* discusses screening library members with a "predetermined macromolecule", cf. column 4, lines 44-51.

Hence, for these reasons alone the combination of the four references does not lead to the claimed invention.

Furthermore, we are unable to find any disclosure in Stemmer et al which pertains to expression/detection/identification of T-

cell epitopes relevant for present claim 25 or which pertains to identification of RNA/peptides which up- or down-regulate expression of cell-surface proteins.

The expression products produced according to Stemmer *et al* are peptides suitable for affinity interaction screening, cf. e.g. the abstract. It cannot be denied that a peptide produced according to Stemmer et al potentially and accidentally could be a T-cell epitope or a regulator of surface protein expression, but these possibilities are not mentioned in Stemmer et al. In fact, the only place we can identify in Stemmer et al where T-cell related subject matter is disclosed is on page 10, lines 21-29, where the murine and human CD4 genes are used to exemplify "cognate genes". There is, however, no indication in that particular paragraph which indicates that the method of Stemmer et al is suitable for identification of T-cell epitopes (claim 25) or RNA/peptides which up- or down-regulate expression of cell surface proteins (claim 26).

Therefore, on reading the disclosure by Stemmer et al, the skilled person would find no guidance or incentive to modify the method disclosed in Kay et al (optionally combined with Wong et al and/or Burke et al) so as to identify T-cell epitopes or peptides/RNA which up- or down-regulate expression of cell surface proteins. Reconsideration and withdrawal of the rejection is respectfully requested.

(4) Rejection of Claim 7 under 35 U.S.C. § 102(e) as being anticipated by Lund et al.

Applicants hereby submit a Declaration under 35 U.S.C. § 1.132. Withdrawal of the rejection is respectfully requested.

In view of the above amendments and submissions it is Applicants' submission that the above-captioned application is now in condition for allowance. In the event there are any issues that can be expedited by telephone conference the Examiner is invited to call the undersigned at the number indicated below.

Respectfully submitted,

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